Mod 2 Day 3: Analysis & Planning I

10/18/2013

1. **Pre-lab Discussion**
2. Tissue Culture: Seed cells for Mod 2 Day 4
3. Sequencing Analysis
4. Paper Discussion

Announcements

- Mod 2 Day 5 presenters **MUST** sign up for a paper today
- Mod 2 Report outlined on the Wiki
- Quiz next time, but no pre-lab discussion
- Watch Shannon's video demonstration before Mod 2 Day 4

Tissue Culture: Media components

1959

Orange/Red ← = YAY!

Trypsin neutralizer

McCoy's 5A Medium

- Vitamins
- Amino Acids
- pH buffers
  - Sodium Bicarbonate
  - HEPES
- pH indicator
  - PHENOL RED
    - Yellow → acidic, bacteria
    - Purple → basic, fungus
- Glutamine! *

NEAA

Non-Essential Amino Acids

- Cocktail
- Glycine
- Alanine
- Growth + Viability

Sodium Pyruvate

- Carbon source for glycolysis
- High metabolic activity

FBS

Fetal Bovine Serum

- GF
- Cytokines
- Lipids
- Signalling for prolif.
  - EGF!

Serum Starve

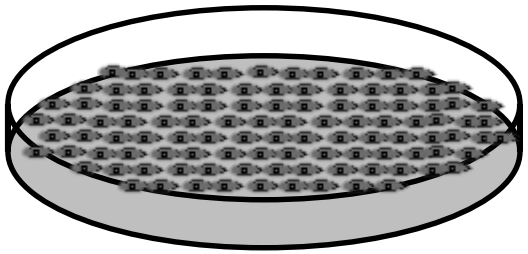
Tissue Culture checklist

1. Media Color → pH
   - Infection
   - Metabolism
2. Sight & Smell

3. Microscope
   - Morphology
   - Attachment
4. Confluency
   % of SA covered → est

Tissue Culture: M2D3 Procedure

SKOV3



LOOK

Trans. to 15mL Conical tube

-Add 8.5mL media

1.5mL Trypsin EDTA

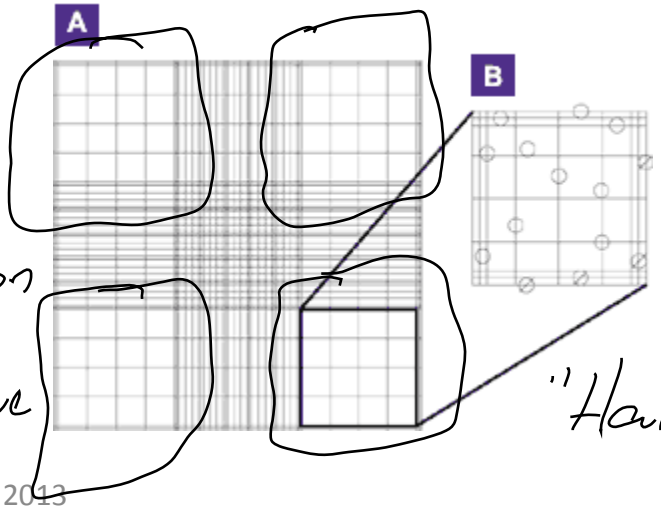
↳ incubate 7-10 mins

Count!

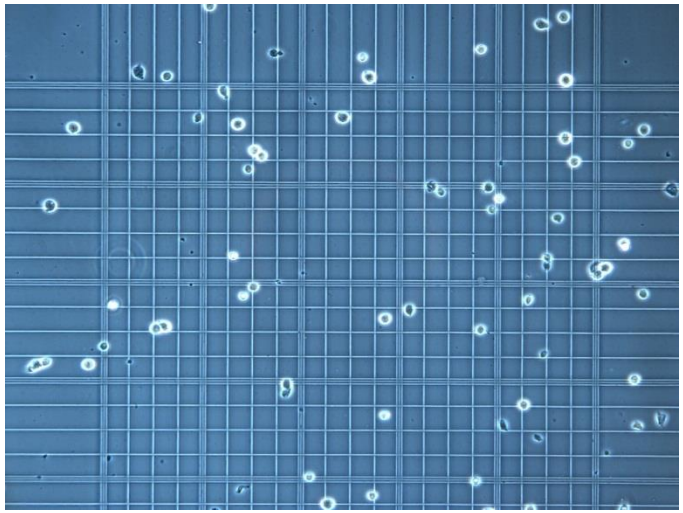
18µL cell suspension

+

2µL Trypan Blue



"Halo"



Tissue Culture: M2D3 Procedure

Spin →  → Remove supernatant

Resuspend in correct conc.

Seeding Density:

Want: 21,000 cells/cm^2

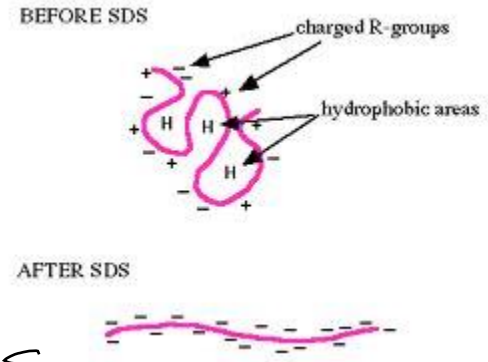
Well surface area: 9.5 cm^2

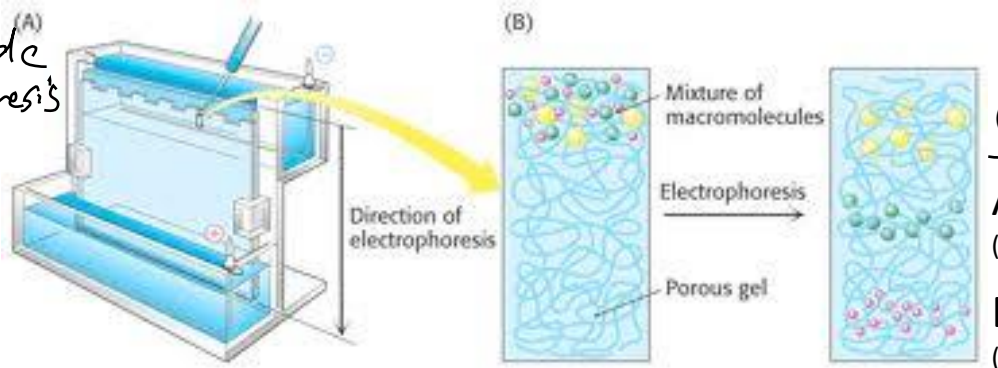
Checklist:

1. Well mixed cells
2. Distribute within wells
3. 4 hour serum starvation before experiment

Mod 2 Day 4: Western Blot

1. Lyse cells
   - Break open (aggressively)
   - Detergent
2. Protein Assay → FNT
   20/30µg protein
3. SDS-PAGE
   - Detergent
   - Polyacrylamide Gel Electrophoresis
     - Charge & conformation neutralized
     - Sorted by Length! Size!





EGFR (~150kDa)

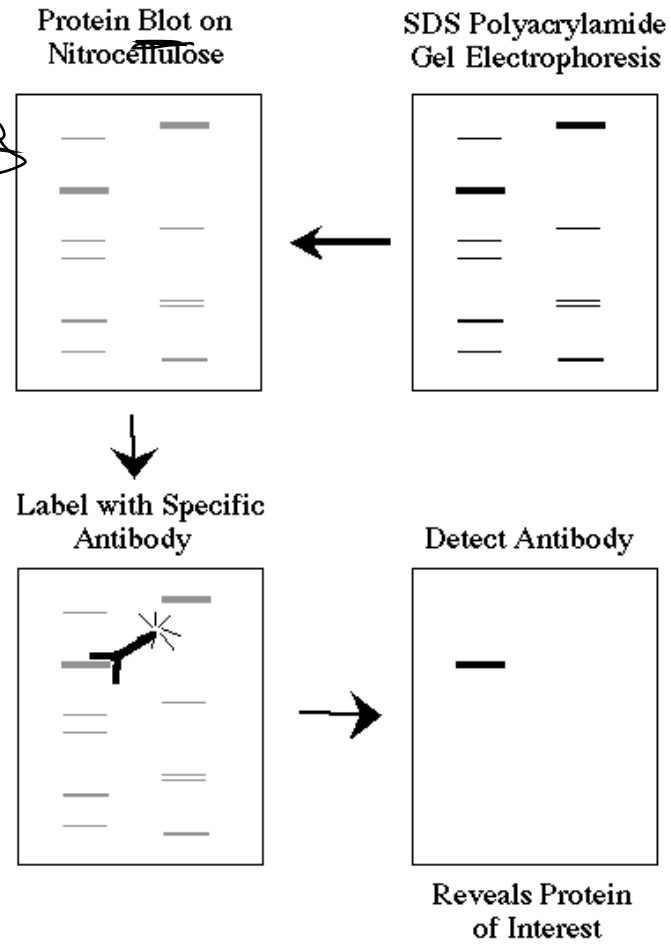
Akt (~64kDa)

Erk (~44kDa)

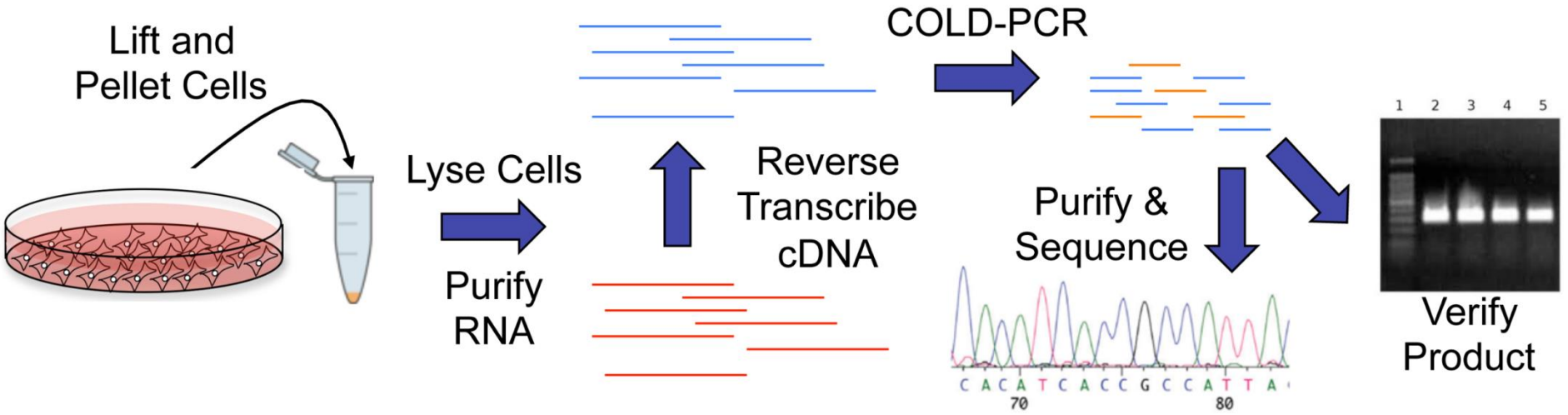
Mod 2 Day 4: Western Blot

4. Nitrocellulose Transfer
   Gel to mmb
   - Flexible

5. Probe w/ Antibodies
   EGFR, Akt, Erk
   ↳ primary ab
   - Secondary Ab
   ↳ 2 wavelengths
   Phosphated vs Normal



Mutation Analysis



Positive Control: HCC-827 NSCLC (Exon 19) On Talk page

Negative Control: MDA-MB231 BC

Experiment: SKOV3 → 19
→ 21

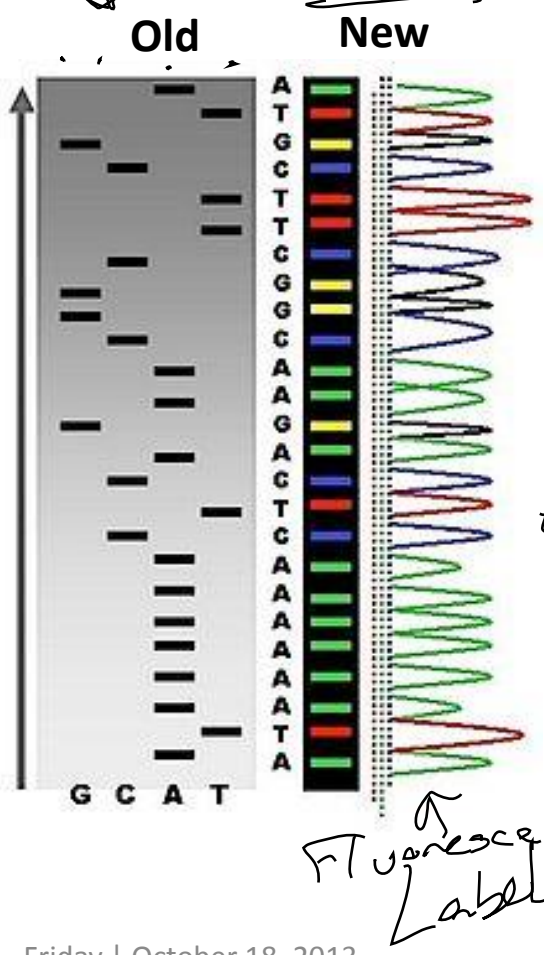
1st $ Last SB → Poor quality!

Sanger Sequencing overview

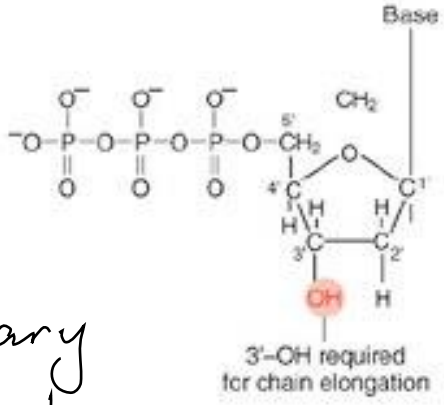
Four dye-labelled dideoxyneucleotides added ~~INSTEAD~~ with of dNTPs

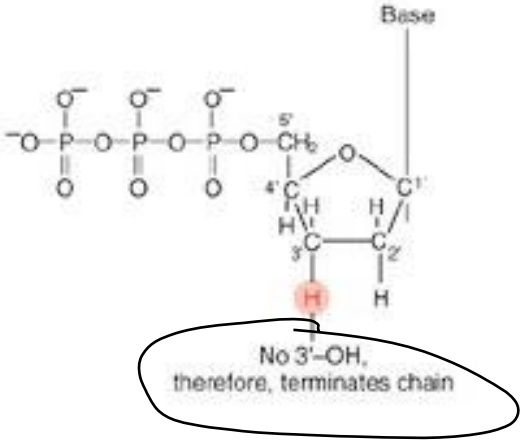
Agarose gel resolve

Genewiz



Capilary Electrophoresis





"Chain terminating reaction"

→ DNA polymerase stops DNA extension w/ di-dNTP

4 RXN's → sequencing

A, T, G, C w/ DNA polymerase

Fluorescently Labeled

Today in lab:

- ~~Red, Orange, Yellow, Green~~ teams:

  Tissue Culture

- Blu~~e, Pink, Purple, and White~~ teams:

  Sequencing

- Paper discussion at **3:30**
- Next time: busy!
  - Stimulate & lyse cells, measure protein conc, run SDS-PAGE
  - Today's FNT will save time!
  - Watch Shannon's discussion video

Announcements

- W/F sequencing rxns no good ☹
  - thoughts on why
  - plan going forward
- BL2 reminders
- Quiz next time at **1:05** sharp (long day!)
- Figures for today's discussion
  - In FC Dropbox or to 20109.talk AT gmail.com
- Switch who goes to TC first: O/Y/G/B